

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Nobuhiro Umeda, et al.

Serial No.: 10/552,015

Group Art Unit: 1625

Filed: October 11, 2005

Examiner: Oh, Taylor V

For: DIAMINE DERIVATIVE, PRODUCTION PROCESS THEREFOR AND
ANTIOXIDANT

DECLARATION UNDER 37 CFR §1.132

Commissioner for Patents

P.O. Box 1450

Alexandria, VA 22313-1450

Dear Sir:

I, Seiichi Uchida, hereby declare and state that:

1. I am a citizen of Japan, residing at Higashi-koiso
Oiso-machi, Naka-gun, Kanagawa, 255-0004, Japan.

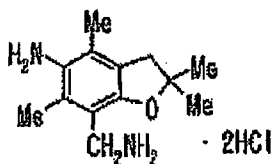
2. I am one of the co-inventors of the U.S. patent application
identified above, and I am fully familiar with the subject matter
thereof as well as the references relied upon by the Examiner
in the prosecution of this application.

3. I obtained a Master's degree from in department of pharmaceutical sciences from Nagoya City University in March, 1982, where I studied the zymology of the lysosomal ATPase of chicken's liver.

4. I am currently employed by Nippon Soda Co., Ltd., and began working for Nippon Soda Co., Ltd., in April 1982, whereat I have engaged in research and development relating to drug discovery.

5. I conducted the following tests in order to evaluate antioxidative effects of compounds disclosed in Ohkawa et al (J. Med. Chem. 1997, 40, 559-573: hereinafter, abbreviated as D1) and Aono et al (EP 0483772 A1: hereinafter, abbreviated as D2).

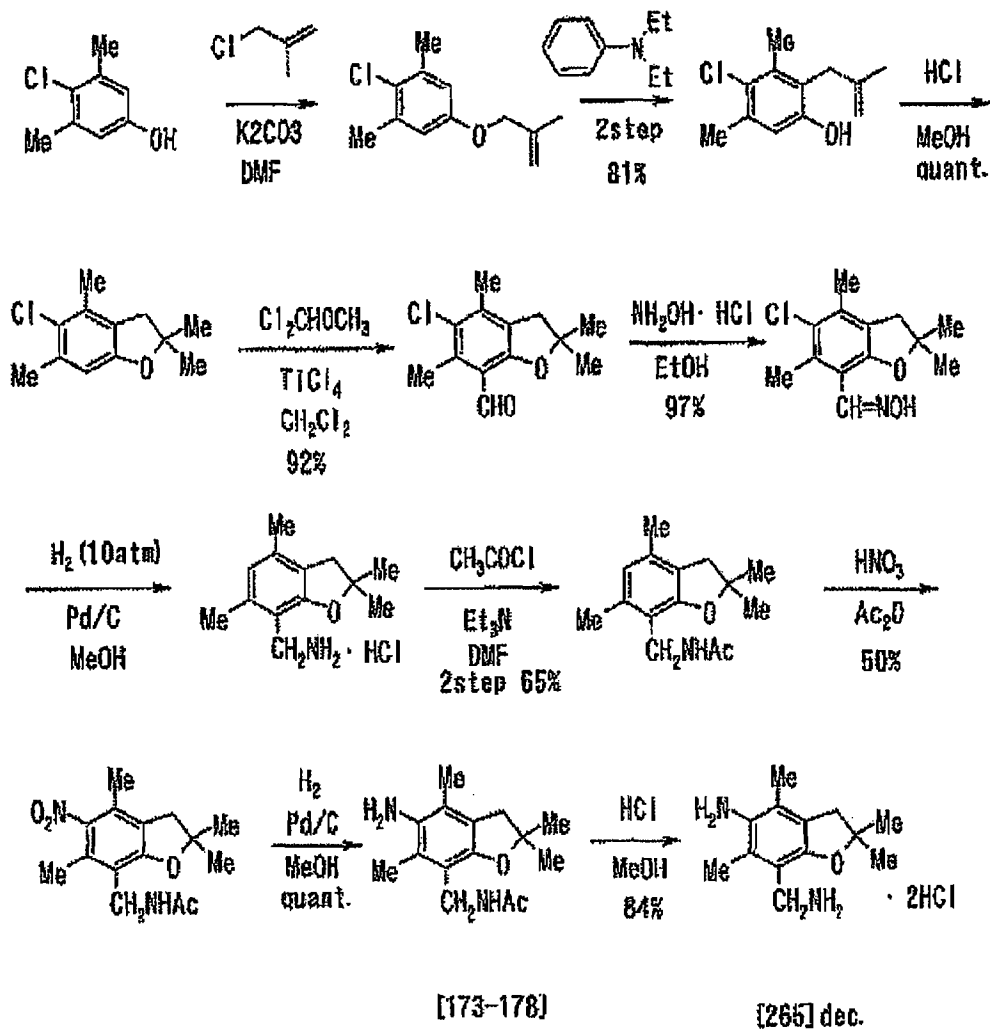
(I-1) Synthesis of Compound A represented by the following formula.



(Method)

Compound A was synthesized in accordance with the following

procedure.



(I-2) Evaluation of Compound A in terms of antioxidative action *in vitro*.

(Method)

The antioxidative action on lipids *in vitro* of Compound A was evaluated by measuring the lipid peroxide activity in a rat brain homogenate. Namely, the rat brain was removed

followed by the addition of five volumes of an aqueous solution of phosphate buffered saline (pH 7.4) (hereinafter, abbreviated as PBS) to the brain while cooling with water, homogenizing with a Teflon homogenizer, and centrifuging for 20 minutes at 10,000 g to collect the supernatant as a brain homogenate. 500 μ M cysteine, 5 μ M ferrous sulfate, Compound A, and 100 mM KCl were added to the resulting brain homogenate followed by incubating for 30 minutes at 37°C and measuring the malondialdehyde that formed due to decomposition of lipid peroxide using the thiobarbituric acid method. The concentration of Compound A required to reduce the amount of lipid peroxide formed by 50% (hereinafter, abbreviated as IC₅₀) was determined from the measured values.

(Result)

The IC₅₀ was more than 10 μ M. Thus, Compound A exhibited poor antioxidative action *in vitro*.

(I-3) Evaluation of Compound A in terms of migration into the brain.

(Method)

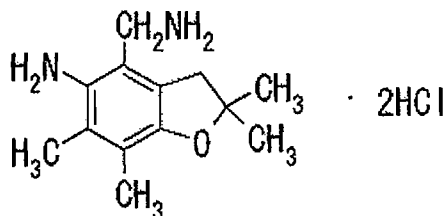
The migration of Compound A into the brain was evaluated by measuring the antioxidative action on lipids *ex vivo*. Compound A dissolved in an aqueous physiological saline solution was intraperitoneally administered to male SD rats (age: 6 weeks) (purchased from Japan SLC) in groups of 3 animals each at a dose of 100 mg/kg. The animals were killed by exsanguination

by severing the carotid artery 30 minutes after administration followed by removal of the brain. The lipid peroxide activity of homogenates of the brain was measured using the method described in paragraph (I-2). The percent inhibition of lipid peroxide formation by Compound A in the brain was determined from the amounts of lipid peroxide formed in a control group (physiological saline dose group) and the test compound group.

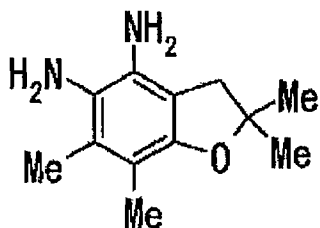
(Result)

The percent inhibition was 8%. Thus, Compound A did not sufficiently cross the blood-brain barrier to migrate into the brain.

(II-1) Comparison of Compound 1 represented by the following formula:



and Compound 11 represented by the following formula:



in terms of antioxidative action on the retina *ex vivo*.

(Method)

Each compound dissolved in an aqueous physiological saline solution was orally administered to male SD rats (age: 6 weeks) (purchased from Japan SLC) in groups of 3 animals each at a dose of 100 mg/kg. Each of the rats was killed and both eyes thereof were removed one hour after the administration. Then, retinas thereof were separated while cooling with ice. The retinas were homogenized and the lipid peroxide activity thereof was measured in the same way as that of the paragraph (I-2). The percent inhibition of lipid peroxide formation by Compound 1 or 11 in the retina was determined from the amounts of lipid peroxide formed in a control group (administered physiological saline free from any test compounds) and each compound group.

(Result)

The percent inhibition of Compound 1 was 61%. The percent inhibition of Compound 11 was 0. Thus, Compound 1 could migrate into the retina. In contrast, Compound 11 could not migrate into the retina.

CONCLUSION

As shown in the above, the compounds disclosed in D1 and D2 exhibited significantly less antioxidative action in comparison with the compound represented by formula (1) of the present invention.

6. I understand fully the content of this declaration.

7. I, Seiichi Uchida, the undersigned declarant further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements are made with the knowledge that willful false statements and the like so made are punishable by fines or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 1 day of September, 2008.

Seiichi Uchida

(Seiichi Uchida)